REMARKS

Claims 72-78, 82-84, and 104-108 were previously pending in this application. Claims 72-73, 84, and 104 have been amended. As a result, claims 72-78, 82-84, and 104-108 are pending for examination, with claims 72 and 104 being independent claims.

Claims 72-73 84, and 104 have been amended to recite that the transfected immortalized somatic cells are "immortalized" somatic cells. The application supports this amendment at, *inter alia*, page 37, lines 27-29, where the transfected cells used in the examples are distinguished from "primary cells." Those transfected cells used in the examples are "[c]ultured mouse Ltk$^-$ fibroblasts"—an immortalized cell line. [Page 40, lines 5-7.] Thus, no new matter has been added.

Provisional Obviousness-type Double Patenting Rejection Over Claims of Application Serial No. 08/461,292

The Examiner provisionally rejected all the pending claims over the pending claims of co-filed Application Serial No. 08/461,292. [Paper No. 20 at p. 2.] Applicant intends to file a terminal disclaimer over Application Serial No. 08/461,292 upon withdrawal of the remaining rejections. Because this is a provisional rejection, Applicant need not address it further at this time.

Provisional and Actual Obviousness-type Double Patenting Rejection Over Claims of Later-filed Applications

The Examiner provisionally and actually rejected all the pending claims over certain claims from later-filed applications and/or the resulting patents, specifically, Application Serial No. 09/549,200, Patent No. 6,303,379, Patent No. 6,048,729, and Patent No. 6,054,288. [Paper No. 20 at pp. 2-13.] Applicant notes that, as the Examiner indicates [*e.g.*, page 4, lines 12-13; page 6, line 11; page 9, lines 4-5; page 11, lines 13-17], the claims of these later filed applications and the resulting patents specifically recite that the transfected cells are either primary cells or secondary cells. Applicant has amended all the pending claims to recite that the transfected immortalized somatic cells are immortalized somatic cells.

The Examiner has conceded that two-way obviousness is required to support the double patenting rejections over the later-filed applications and resulting patents. [Paper No. 20 at p. 13,

lines 9-12.] Thus, these rejections must be withdrawn if either the pending claims are nonobvious over the claims of the later-filed applications and resulting patents, or the claims of the later-filed applications and resulting patents are nonobvious over the pending claims. "If **either** analysis does not compel a conclusion of nonobviousness, no double patenting rejection of the obvious-type is made . . . . " M.P.E.P. § 804 at p. 800-23, col. 2 (emphasis added).

The double patenting rejections should be withdrawn because the later-filed primary or secondary cell claims would not have been obvious over the earlier-filed immortalized cell claims pending in this application. One of ordinary skill in the art familiar with such gene transfer to a recipient subject with immortalized cells would not have been motivated to attempt it with primary or secondary cells. Moreover, even if such motivation did exist, there would have been no reasonable expectation of success. Thus, these provisional and actual obviousness-type double patenting rejections should be withdrawn.

One of ordinary skill in the art familiar with such immortalized cell work would not have been motivated to attempt it with primary or secondary cells because primary and secondary immortalized somatic cells are very different from immortalized cells. These differences would have made primary and secondary cells less desirable to one of ordinary skill in the art than immortalized cells. For example, primary and secondary cells have finite life spans in culture, where growth generally slows and arrests after about 50 to 90 mean population doublings. In addition, over time, primary and secondary immortalized somatic cells generally exhibit an increase in cell doubling time and cell volume, and a decrease in saturation density and senescence. In contrast, immortalized cells in culture are generally characterized by a shorter doubling time, decreased cell volume, and increased saturation density. It is critical to note that immortalized cells exhibit an essentially unlimited capacity to divide *in vitro*; that is, they do not senesce. Because these characteristics would have made immortalized cells more desirable than primary or secondary cells for transferring a gene to a recipient subject, one of ordinary skill in the art would not have been motivated to substitute primary or secondary cells for immortalized cells.

Even if one of ordinary skill in the art would have been motivated to substitute primary or secondary cells for immortalized cells, one of ordinary skill in the art would not have had a reasonable expectation of success because primary and secondary cells handle DNA in a

fundamentally different manner than immortalized cells handle DNA. Immortalized cells are more susceptible to mutation and have a decreased ability to repair DNA. [Tsujimura et al., *Proc. Natl. Acad. Sci. USA* 87:1566-1570, 1990 (copy enclosed); McGregor et al., *Immortalized somatic Cell and Molecular Genetics* 17:463-469, 1991; (copy enclosed).] Also, while telomeres shorten during the aging of a normal diploid cell, telomeres remain the same length in immortalized cells. [Harley et al., *Nature* 345:458-460, 1990 (copy enclosed).] In fact, immortalized cells possess a novel enzyme, telomerase, that prevents telomere shortening by adding DNA to the ends of chromosomes. Thus, the cellular processing of DNA in primary and secondary cells is fundamentally different from that in immortalized cells.

Holliday [*TIG* 5(2):42-45, 1989 (copy enclosed)] presents evidence of the differences between primary and secondary cells on the one hand and immortalized cells on the other hand. For example, it teaches that immortalized cells and primary and secondary cells handle DNA in dramatically different ways, with the genome of normal cells being much more stable than that of immortalized cells. For example, Holliday discusses differences in karyotype and the frequencies of non-disjunction of chromosomes, chromosome rearrangements, gene amplification, DNA methylation, and integration of foreign DNA between immortalized and primary or secondary immortalized somatic cells. Regarding integration of foreign DNA, Holliday states, at Page 44, Table 1, footnote d:

> Several laboratories have failed to obtain stable transfectants with DNA integrated into a chromosome. (For obvious reasons, these negative results remain unpublished.) An illustration of this comes from a comparison of human diploid fibroblasts, strain MRC-5, and its SV40-transformed derivative. Both cells take up exogenous DNA, but stable transfection is very rare in the diploid parent, whereas it is frequent in the transformed derivative (L.I. Huschtscha, pers. commun.) However, DNA can be integrated in the chromosomes of eggs or embryonic cells, and into immortalized somatic cells using retrovirus vectors.

Chromosome non-disjunction and rearrangement events in immortalized cells, which are generally tumorogenic, and primary cells are compared at page 42 by Holliday as well:

> Many tumour cell lines are heteroploid with a continually varying chromosome number. In such cells, there is probably at least one abnormal chromosome segregation per division, and there may be several. The overall frequency of nondisjunction is likely to be at least 100-fold higher than that of normal diploid cells. Some transformed or tumour

> cells have been reported to have diploid, or quasi-diploid karyotypes, but such populations tend to give rise to hypo- or hyper-diploid cells. The pseudo-diploid Chinese hamster ovary (CHO) cell line has been used extensively in immortalized somatic cell genetics, and appropriate markers make it possible to measure chromosome nondisjunction. In hybrids heterozygous for two X chromosome-linked markers, abnormal segregation occurred at rates of 1.4 - 3.0 x 10^{-3} per cell division in different experiments, which is almost certainly considerably higher than the rate in diploid cells.

While transformed, immortalized cells experience a high frequency of chromosome nondisjunction, normal diploid cells experience a low frequency. [Holliday, Table 1.] These events demonstrate the substantial differences in the abilities of non-immortalized and immortalized cells to repair DNA damage and to maintain a stable genome.

As exemplified by these fundamental genetic differences between immortalized and primary or secondary immortalized somatic cells, Holliday clearly shows that the genomes of normal cells are more stable than those of immortalized cells. Holliday shows that, at the time the presently claimed invention was made, immortalized cells were known to possess a "plastic" genetic structure that is easily manipulated. In contrast, primary and secondary cells simply were not expected to process DNA in the same manner as immortalized cells, and thus would not have been expected to be capable of undergoing stable transfection based on observations of immortalized cells.

An article by Mes-Mason [*J.Cell Sci.* 94:517-525, 1989 (copy enclosed)] even more clearly illustrates that one of ordinary skill in the art would not have had a reasonable expectation of success in substituting primary or secondary cells for immortalized cells to transfer a gene to a recipient subject. Like the Holliday article, the Mes-Mason article reports a failure in obtaining stably transfected primary cells. More importantly, the Mes-Mason article reports that this failure was remedied by immortalizing the primary cells. Thus, "no cell lines could be established after transfection of primary cells with pMTONCO DNA alone," but "[g]reater than 50% of primary cells isolated after transfection with the pSV2NEOSVEB1a plasmid could sustain growth in culture." [page 522, columns 1-2.] Thus, Mes-Mason was not able to isolate cell lines derived from stably transfected cells until those cells were immortalized with polyomavirus.

Accordingly, one of ordinary skill in the art would not have been motivated to substitute primary or secondary cells for immortalized cells to transfer a gene to a recipient subject. Even if such a motivation would have existed, one of ordinary skill in the art would not have had a reasonable expectation of success in susbtituting primary or secondary cells for immortalized cells. The obviousness-type double patenting rejections over the later-filed applications and resulting patents should, therefore, be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, this application should now be in condition for allowance. A notice to this effect is respectfully requested. If the Examiner believes, after this amendment, that the application is not in condition for allowance, the Examiner is requested to call the Applicant's attorney at the telephone number listed below.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

Respectfully submitted,
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